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Vasopressin Does Not Mediate the Inhibition of Ethanol Drinking by the Renin-Angiotensin System

A. D. ROSS, E. PERLANSKI AND L. A. GRUPP[1]

Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8 and Neurobiology Department, Addiction Research Foundation of Ontario 33 Russell St., Toronto, Ontario, Canada M5S 2S1

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ROSS, A. D., E. PERLANSKI AND L. A. GRUPP. *Vasopressin does not mediate the inhibition of ethanol drinking by the renin-angiotensin system.* PHARMACOL BIOCHEM BEHAV 36(4) 761–765, 1990.—Manipulations which are known to enhance activity in the renin-angiotensin system (RAS) have been found to reduce the voluntary consumption of ethanol in rats. Since angiotensin II is a potent stimulus for the release of vasopressin (VP), it is possible that the RAS modulates ethanol (ETOH) consumption through a mechanism involving VP. The present investigation examined the effect of peripheral injections of arginine-VP (AVP) and desglycinamide-AVP (DGAVP) on ETOH consumption in rats given daily one-hour access to ETOH. Daily subcutaneous treatment with AVP or DGAVP had no effect on ETOH consumption at doses ranging from 2 to 200 μg/kg (SC). Blood pressure was substantially elevated following a single 20 μg/kg injection of AVP, indicating that AVP was biologically active at doses which failed to alter ethanol consumption. These findings indicate the VP does not affect established ETOH drinking and furthermore is not likely a critical factor in the reduction of ETOH intake by the RAS.

Ethanol drinking Angiotensin II Arginine[8]-vasopressin Desglycinamide[9]-arginine[8]-vasopressin Renin-angiotensin system

RESEARCH from our laboratory has demonstrated that the voluntary consumption of ethanol (ETOH) can be altered by manipulations which change activity in the renin-angiotensin system (RAS). For example peripheral injections of angiotensin II (ANG II) or angiotensin III (ANG III), substantially reduce the intake of ethanol (ETOH) in rats (6,20). Other experiments using surgical (7), dietary (9,10) and drug (8,23) treatments to alter RAS activity have consistently shown an inverse relationship between RAS activity and alcohol intake. The mechanism underlying this effect is not yet clearly understood and the possibility exists that the ANG II-mediated reduction in ethanol intake may involve other systems which are stimulated by the RAS (21). For example, ANG II has been shown to stimulate the release of vasopressin (VP) from the posterior pituitary (18) and like ANG II, VP is involved in the hormonal regulation of fluid balance and may contribute to the pressor effect of ANG II (11). Activity in VP-sensitive neurons of the paraventricular nucleus appears to be enhanced by stimulation of ANG II-sensitive neurons in the subfornical organ (13,24) and area postrema (1), which are critical sites for the dipsogenic and central pressor effects of ANG II. These findings indicate that ANG II can modulate the functional effects of VP. Furthermore, the release of VP is altered following ETOH consumption in humans (4) and animals (2) and VP has been shown to maintain ETOH tolerance and to affect the acquisition of ETOH drinking behavior (5, 12, 16). Taken together, this evidence suggests that, like ANG II, VP may also play a role in controlling ETOH drinking behavior and that the reduction of ETOH intake in rats treated with ANG II could occur as a result of enhanced VP release. The following experiments investigate this possibility by examining the ability of peripherally administered VP to alter ETOH consumption in rats.

METHOD

Subjects

Seventy naive male Wistar rats (Charles Rivers, Montreal) weighing between 225 and 250 g were housed individually and maintained on a reverse 12/12 hour light/dark cycle with lights off at 7:00 a.m. The rats had ad lib access to Purina Rat Chow No. 5001 (0.4% sodium), except during daily one-hour drinking sessions. Water was available continuously in the home cage.

Drug Preparation

Solutions of 3 and 6% (w/v) ETOH were prepared in tap water.

[1]Requests for reprints should be addressed to Dr. Larry A. Grupp, Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

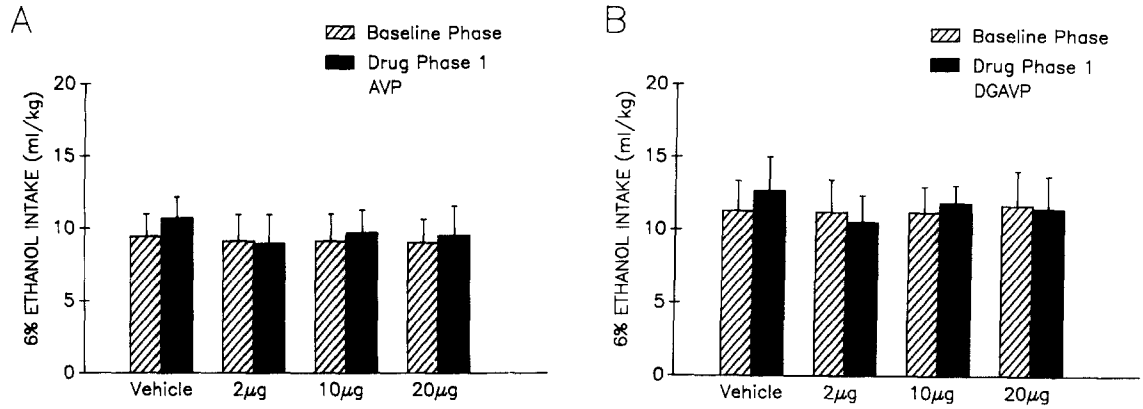

FIG. 1. Mean daily intake of 6% ethanol over one-hour drinking sessions during the Baseline Phase and during treatment (Drug Phase 1) with (A) AVP or (B) DGAVP. Bars represent standard error of the mean.

AVP and DGAVP (Bachem) were dissolved in 0.1% acetic acid and diluted to the desired concentration in 0.9% saline at a pH of 6.5–7.0. Drug solutions were administered subcutaneously (SC) in a volume of 1 ml/kg body weight. Fresh drug solutions were prepared each test day.

Apparatus

Daily drinking sessions were conducted in standard hanging wire cages (30 × 20 × 15 cm) equipped with two 17-ml graduated tubes mounted on the front of each cage. The equipment used for measuring blood pressure consisted of an inflatable rubber cuff with a Pneumatic Pulse Transducer (Narco Biosystem, Houston) linked to a Narco Biosystem PE-300 Programmed Electro-Sphygmomanometer and a Narcotrace 40 polygraph recorder. A Plexiglas cylinder equipped with a heating pad set at 37°C was used to restrain the animals and maintain body temperature during recording.

Limited Access Procedure

The rats in this experiment were offered alcohol using the limited access procedure (14,15): each day during the dark cycle (six hours after lights off) the rats were removed from their home cages, weighed, and then transferred for one hour to individual drinking cages equipped with two tubes, one containing ETOH, the other containing tap water. The positions of the two tubes were alternated daily and no food was available in the drinking cage. After the hour elapsed, the amounts of alcohol and water consumed were recorded and the animals were returned to their home cages. Using this procedure the animals were initially offered a choice between a relatively low alcohol concentration (3% w/v) for 10 consecutive days followed by a choice between 6% (w/v) alcohol and water for a further 14 days during which intake stabilized. This latter period was considered the baseline phase since the effect of vasopressin administration was to be tested on alcohol consumption using the 6% concentration.

Drug Phase 1

Following the baseline phase, the rats were randomly assigned to one of eight groups and matched for 6% ETOH intake during the baseline phase. Three groups received subcutaneous (SC) injections of AVP at doses of 2, 10, and 20 μg/kg while the other three groups received SC DGAVP at doses of either 2, 10 or 20 μg/kg (n = 9 per group). The two remaining groups served as controls for the AVP and DGAVP groups and received injections of vehicle (n = 8 per group). All injections were given immediately prior to access to ETOH using the limited access procedure. The concentration of ETOH was maintained at 6% (w/v) for 7 consecutive days of drug testing.

Drug Phase 2

The two groups of rats that previously received 20 μg/kg of AVP or DGAVP were given the same drug but at a greatly increased dose of 200 μg/kg, immediately prior to access to 6% ETOH, for a further 7 days. The two respective control groups continued to receive injections of vehicle.

Blood Pressure Measurement

After a 7-day drug-free interval, rats that had previously received 2 μg/kg of AVP (n = 9) or DGAVP (n = 9) during Drug Phase 1, received a single injection of vehicle and the mean arterial blood pressure (BP) was calculated. The following day, the two respective groups were given SC injections of 20 μg/kg AVP or DGAVP and BP was measured again. Blood pressure (BP) was measured from the tail artery every minute over a 10-minute interval immediately following injection and the average BP was calculated.

Statistical Analysis

An analysis of variance (ANOVA) was used to determine group differences in fluid consumption and BP. Post hoc analyses were carried out using Duncan's tests at a significance level of $p<0.05$. Paired comparisons were made using two-tailed paired *t*-tests.

RESULTS

Drug Phase 1

Figure 1A and B show mean ETOH consumption over the 10-day period prior to drug treatment (Baseline Phase) and during the first 7 days of treatment (Drug Phase 1) with AVP or DGAVP, respectively. A three-way ANOVA revealed nonsignificant effects of Drug, $F(1,48) = 1.82$, n.s., Dose, $F(2,48) = 0.41$, n.s., and Phase, $F(1,48) = 0.44$, n.s. Nonsignificant interactions were found

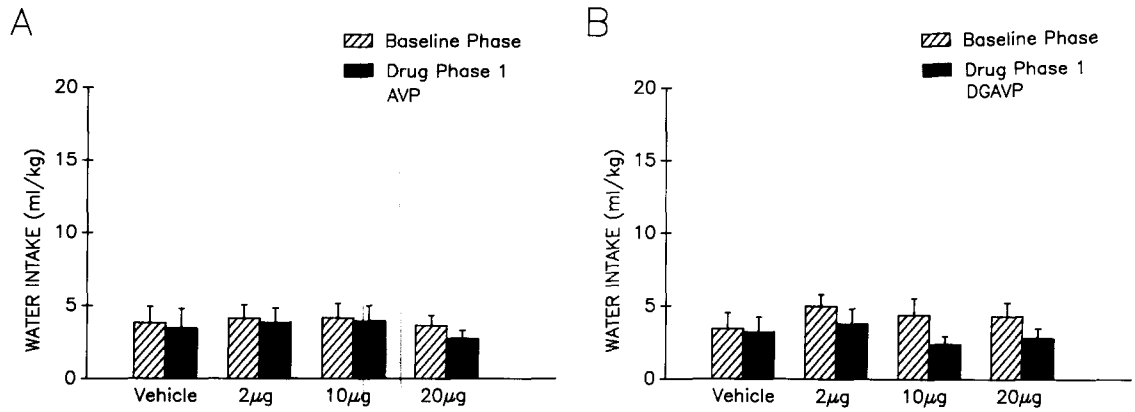

FIG. 2. Mean daily intake of water over one-hour drinking sessions during the Baseline Phase and during treatment (Drug Phase 1) with (A) AVP or (B) DGAVP. Bars represent standard error of the mean.

between Drug and Dose, $F(2,48)=0.01$, n.s., Drug and Phase, $F(1,48)=0.18$, n.s., Dose and Phase, $F(2,48)=0.49$, n.s., and Drug, Dose and Phase, $F(2,48)=0.07$, n.s. Thus, ETOH intake was completely unaffected by treatment with either AVP or DGAVP at the range of doses tested in Drug Phase 1. ETOH consumption was also unchanged in the AVP, $t(7)=1.643$, n.s., and DGAVP, $t(7)=0.852$, n.s., control groups. Figure 2A and B show mean water intake prior to and during treatment with AVP or DGAVP, respectively. A three-way ANOVA showed nonsignificant effects of Drug, $F(1,48)=0.00$, n.s., and Dose, $F(2,48)=0.40$, n.s., and nonsignificant interactions between Drug × Dose, $F(2,48)=0.22$, n.s., Dose × Phase, $F(2,48)=0.31$, n.s., and Drug × Dose × Phase, $F(2,48)=0.47$, n.s. The effect of Phase, $F(1,48)=15.73$, $p<0.001$, and the interaction of Drug × Phase, $F(1,48)=4.53$, $p<0.05$, were significant. Thus, at certain doses, drug treatment produced a decrease in water intake. Post hoc analysis revealed nonsignificant changes in water intake at all doses of AVP, at 2 μg/kg of DGAVP and in the AVP, $t(7)=0.806$, n.s., and DGAVP, $t(7)=0.738$, n.s., control groups. Doses of 10 and 20 μg/kg DGAVP resulted in a small but significant decrease in water intake ($p<0.05$). This finding was unexpected since DGAVP reportedly lacks peripheral pressor and antidiuretic activity (3).

Drug Phase 2

Figure 3 shows mean fluid consumption prior to and during treatment with 200 μg/kg of AVP (Fig. 3A) or DGAVP (Fig. 3B). Statistical analysis showed nonsignificant effects of Drug, $F(3,30)=0.43$, n.s., and Phase, $F(1,30)=0.32$, n.s., and a nonsignificant interaction between Drug × Phase, $F(3,30)=0.03$, n.s., indicating that AVP and DGAVP had no effect on the intake of ETOH. A two-way ANOVA of water intake showed nonsignificant effects of Drug, $F(3,30)=0.04$, n.s., and Phase, $F(1,30)=2.18$, n.s., and a nonsignificant interaction between Drug × Phase, $F(3,30)=0.97$, n.s., indicating that water intake was unaffected by treatment with AVP or DGAVP.

Blood Pressure

Figure 4 shows mean BP in rats before and during treatment with 20 μg/kg of AVP or DGAVP. A two-way ANOVA revealed

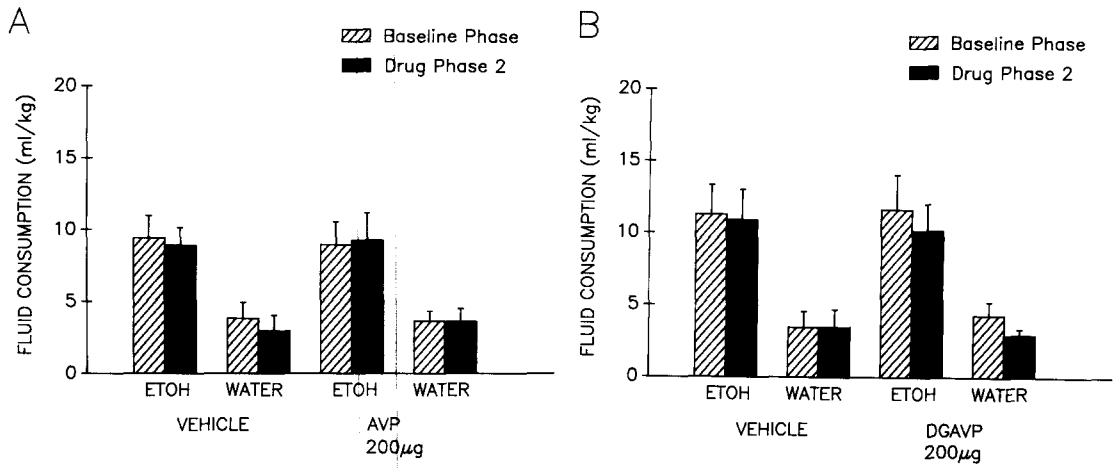

FIG. 3. Drug Phase 2—Mean daily intake of 6% ethanol and water over one-hour drinking sessions during the Baseline Phase and during treatment with 200 μg/kg (Drug Phase 2) of (A) AVP or (B) DGAVP. Bars represent standard error of the mean.

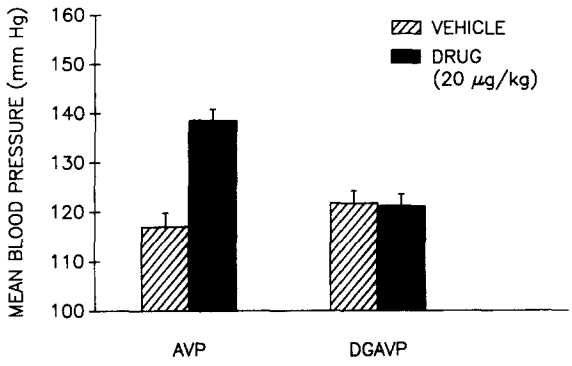

FIG. 4. Mean arterial blood pressure over a 10-minute interval following a single injection of vehicle or 20 μg/kg of AVP or DGAVP. Bars represent standard error of the mean.

a significant effect of Phase, $F(1,16)=49.88$, $p<0.001$, a non-significant effect of Drug, $F(1,16)=3.22$, n.s., and a significant interaction of Drug × Phase, $F(1,16)=3.217$, $p<0.001$, indicating that drug treatment was capable of raising blood pressure, but only in the group receiving AVP ($p<0.05$). This result confirms previous findings which indicate that AVP, but not DGAVP, is capable of eliciting pressor effects (3).

DISCUSSION

This experiment has shown that treatment with AVP or DGAVP does not alter ETOH consumption in rats who have acquired alcohol drinking using the limited access procedure. Several prior studies have shown that VP can modulate ETOH consumption, but only at certain times during the course of development of ETOH drinking behavior. For example, rats treated with 1–4 μg/kg of DGLVP, a VP fragment which lacks antidiuretic activity, consumed higher concentrations of ETOH than control rats, only when the drug was administered during the acquisition of ETOH drinking behavior, but was ineffective when given after ETOH drinking behavior had already been established (5,16). The consumption of both ETOH and water was found to be increased in VP-deficient Brattleboro diabetes insipidus rats, but remained at normal levels if treated with LVP, DGAVP or VP tannate (17,19). These latter investigators also found that VP treatment had no effect on normal rats, nor was there an effect if VP was administered after ETOH drinking behavior had been established. It was concluded that the enhanced intake of ETOH in Brattleboro rats was an epiphenomenon secondary to a polydipsic state resulting from excessive diuresis (19).

The initial purpose of this study was to examine whether VP is capable of altering ETOH intake fostered by the limited access procedure so as to comment on the possibility that enhanced VP release may be responsible for the ability of the RAS to alter ETOH intake. In addition, since both ANG II and AVP are potent pressor agents, we reasoned that if AVP was also capable of altering ETOH intake, pressor activity may possibly be involved in the ability of AVP and ANG II to modulate ETOH consumption. We found that ETOH consumption was not affected by DGAVP which lacks central pressor activity, or by AVP, which at equivalent doses produced a potent pressor effect. Since both ANG II and AVP can elevate BP, but only ANG II is effective in reducing ETOH intake, our results suggest that the reduction of ETOH intake by ANG II does not occur solely as a result of elevated blood pressure. Most studies examining the effect of VP on alcohol intake, alcohol tolerance or memory, work within the 1–20 μg/kg dose range. However, in the present study doses in that range, as well as a dose of 200 μg/kg—ten-fold greater than that required to influence the above noted processes or to produce a significant elevation in blood pressure—still failed to alter ETOH intake. It is therefore unlikely that the dose range of vasopressin examined in the present study was unable to produce vasopressin levels comparable to those produced by angiotensin treatment. Taken together, the present findings show that VP does not alter established ETOH drinking in the limited access procedure and suggest that VP does not play a critical role in the modulation of ETOH intake by the renin-angiotensin system.

In summary, this study has shown that SC administration of either AVP or DGAVP does not alter established ETOH drinking behavior and consequently, that the stimulation of VP release is not a likely factor in the reduction of ETOH drinking by the RAS.

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REFERENCES

1. Bishop, V. S.; Hasser, E. M.; Undesser, K. P. Vasopressin and sympathetic nerve activity: Involvement of the area postrema. In: Buckley, J. P.; Ferrario, C. M., eds. Brain peptides and catecholamines in cardiovascular regulation. New York: Raven Press; 1987: 373–382.
2. Colbern, D. L; ten Haaf, J.; Tabakoff, B.; van Wimersma Greidanus, T. B. Ethanol increases plasma vasopressin shortly after intraperitoneal injection in rats. Life Sci. 37:1029–1032; 1985.
3. DeWied, D. Minireview: Peptides and behavior. Life Sci. 20:195–204; 1977.
4. Eisenhofer, G.; Johnson, R. H. Effect of ethanol ingestion on plasma vasopressin and water balance in humans. Am. J. Physiol. 242: R522–R527; 1982.
5. Finkelberg, F. H.; Kalant, H.; Le Blanc, A. E. Effect of vasopressin-like peptides on consumption of ethanol by the rat. Pharmacol. Biochem. Behav. 9:453–458; 1978.
6. Grupp, L. A.; Killian, M.; Perlanski, E.; Stewart, R. B. Angiotensin II reduces voluntary alcohol intake in the rat. Pharmacol. Biochem. Behav. 29:479–482; 1988.
7. Grupp, L. A.; Perlanski, E.; Leenen, F. H. H.; Stewart, R. B. Voluntary alcohol intake is attenuated in Two-Kidney,One-Clip, but not in One-Kidney,One-Clip Goldblatt hypertensive rats. Alcohol 5:173–179; 1988.
8. Grupp, L. A.; Perlanski, E.; Stewart, R. B. Attenuation of alcohol intake by a serotonin uptake inhibitor: Evidence for mediation through the renin-angiotensin system. Pharmacol. Biochem. Behav. 30:823–827; 1988.
9. Grupp, L. A.; Perlanski, E.; Stewart, R. B. Dietary salt and Doca-salt treatments modify ethanol self-selection in rats. Behav. Neural Biol. 40:239–250; 1984.
10. Grupp, L. A.; Perlanski, E.; Stewart, R. B. Salt restriction and the voluntary intake of ethanol in rats. Physiol. Psychol. 12:242–246; 1986.
11. Hoffman, W. E.; Phillips, M. I. The role of ADH in the pressor response to intraventricular angiotensin II. In: Buckley, J. P.; Ferrario, C. M., eds. Central action of angiotensin and related hormones. New York: Pergamon; 1977:307–314.
12. Hung, C. R.; Tabakoff, B.; Melchior, C. L.; Hoffman, P. L. Intraventricular arginine vasopressin maintains ethanol tolerance. Eur. J. Pharmacol. 106:645–648; 1984.
13. Iovino, M.; Steardo, L. Thirst and vasopressin secretion following central administration of angiotensin II in rats with lesions of the

septal area and subfornical organ. Neuroscience; in press.
14. Linseman, M. A. Alcohol consumption in free feeding rats: Procedural, genetic and pharmacokinetic factors. Psychopharmacology (Berlin) 92:254–261; 1987.
15. Macdonall, J. S.; Marcucella, H. Increasing the rate of ethanol consumption in food- and water-satiated rats. Pharmacol. Biochem. Behav. 10:211–216; 1979.
16. Mucha, R. F.; Kalant, H. Effects of desglycinamide(9)-lysine(8)-vasopressin and prolyl-leucyl-glycinamide on oral ethanol intake in the rat. Pharmacol. Biochem. Behav. 10:229–234; 1979.
17. Myers, R. D.; Critcher, E. C.; Cornwell, N. N. Effect of chronic vasopressin treatment on alcohol drinking of Brattleboro HZ and DI rats. Peptides 4:359–366; 1983.
18. Ramsey, D. J.; Keil, L. C.; Sharpe, M. C.; Shinsako, J. Angiotensin II infusion increases vasopressin, ACTH, and 11-hydroxycorticosteroid secretion. Am. J. Physiol. 234:R66–R71; 1978.
19. Rigter, H.; Crabbe, J. C. Vasopressin and ethanol preference. I. Effects of vasopressin and the fragment DGAVP on altered ethanol preference in Brattleboro diabetes insipidus rats. Peptides 6:669–676; 1985.
20. Ross, A. D.; Perlanski, E.; Grupp, L. A. Attenuation of voluntary ethanol intake in rats by angiotensin II and angiotensin II fragments. Alcohol.: Clin. Exp. Res. 12:304; 1988.
21. Ross, A. D.; Perlanski, E.; Grupp, L. A. Prostaglandin E_2 reduces voluntary ethanol intake in the rat. In press.
22. Grupp, L. A.; Sneddon, B.; Solway, E.; Perlanski, E.; Stewart, R. B. The beta adrenergic agonist isoproterenol suppresses voluntary alcohol intake in rats. Pharmacol. Biochem. Behav. 33:493–495; 1989.
23. Spinosa, G.; Perlanski, E.; Stewart, R. B.; Grupp, L. A. Angiotensin converting enzyme inhibitors: Animal experiments suggest a new pharmacological treatment for alcohol abuse in humans. Alcohol.: Clin. Exp. Res. 12:65–70; 1988.
24. Tanaka, J.; Saito, H.; Kaba, H.; Seto, K. Subfornical organ neurons enhance the activity of paraventricular vasopressin neurons in response to intravenous angiotensin II. Neurosci. Res. 4:424–427; 1987.